

Attorney Docket No.: UT-0006  
Inventors: Rao et al.  
Serial No.: 09/109,858  
Filing Date: July 2, 1998  
Page 2

12. (amended) A method of isolating a pure population of rodent or human CNS neuron-restricted precursor cells comprising the steps of:

(a) isolating a population of rodent or human multipotent CNS stem cells which generate both neurons and glia;

(b) incubating the multipotent CNS stem cells in NEP medium [configured for cells to begin differentiating];

(c) replating the multipotent CNS stem cells on laminin in the absence of chick embryo extract to induce cell differentiation;

[(c)] (d) purifying from the differentiating cells a subpopulation of cells expressing embryonic neural cell adhesion molecules via a procedure selected from the group consisting of specific antibody capture, fluorescence activated cell sorting, and magnetic bead capture; and

[(d)] (e) incubating the purified subpopulation of cells in a FGF-containing medium configured for supporting adherent growth thereof to obtain an isolated, purified population of rodent or human CNS neuron-restricted precursor cells, wherein said neuron-restricted precursor cells [require FGF and] differentiate into CNS neuronal cells upon replacement of adherent growth supporting medium with retinoic acid containing medium and fail to

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Attorney Docket No.: UT-0006  
Inventors: Rao et al.  
Serial No.: 09/109,858  
Filing Date: July 2, 1998  
Page 3

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proliferate or differentiate in astrocyte-promoting medium containing FGF and 10% fetal calf serum [but not into CNS glial cells].

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21. (amended) A method of isolating a pure population of rodent or human CNS neuron-restricted precursor cells comprising the steps of:

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(a) removing a sample of spinal cord tissue from a rodent or human embryo at a stage of embryonic development after closure of the neural tube but prior to differentiation of glial and neuronal cells in the neural tube;

(b) dissociating cells comprising the sample of spinal cord tissue removed from the embryo;

(c) purifying from the dissociated cells a subpopulation expressing embryonic neural cell adhesion molecule;

(d) plating the purified subpopulation of cells in feeder-cell-independent culture on a substratum and in a medium configured for supporting adherent growth of the neuron-restricted precursor cells; and

(e) incubating the plated cells at a temperature and in an atmosphere conducive to growth to obtain an isolated, pure population of neuron-restricted precursor cells, wherein said neuron-restricted precursor cells require FGF for adherent growth.

Attorney Docket No.: UT-0006  
Inventors: Rao et al.  
Serial No.: 09/109,858  
Filing Date: July 2, 1998  
Page 4

B2 [and] differentiate into CNS neuronal cells upon replacement of adherent growth supporting medium with retinoic acid containing medium and fail to proliferate or differentiate in astrocyte-promoting medium containing FGF and 10% fetal calf serum [but not into CNS glial cells].

B3 26. (amended) A pure population of rodent or human CNS neuron-restricted precursor cells isolated by the method of claim 12, wherein said neuron-restricted precursor cells require FGF to support adherent growth, [and] differentiate into CNS neuronal cells upon replacement of adherent growth supporting medium with retinoic acid containing medium and fail to proliferate or differentiate in astrocyte-promoting medium containing FGF and 10% fetal calf serum [but not into CNS glial cells].

B3 27. (amended) A pure population of rodent or human CNS neuron-restricted precursor cells isolated by the method of claim 21, wherein said neuron-restricted precursor cells require FGF to support adherent growth, [and] differentiate into CNS neuronal cells upon replacement of adherent growth supporting medium with retinoic acid containing medium and fail to proliferate or differentiate in astrocyte-promoting medium containing FGF and 10% fetal calf serum [but not into CNS glial cells].